Bacteria

MALOLACTIC FERMENTATION

and much more !

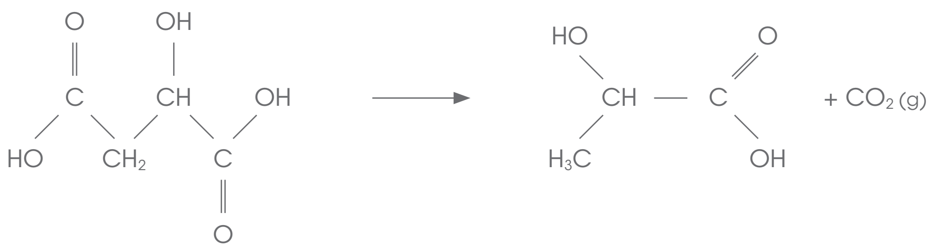
IOC

Révélons votre différence

Once considered as a secondary phase in the winemaking process, malolactic fermentation (MLF) of wines was for a long time left to chance. Today, its impacts and importance, which go well beyond just transforming malic acid into lactic acid, are well-known and acknowledged. MLF is now an essential stage in the winemaking and ageing process, and has bearing on the rapid availability of the wine and how work is organised in the cellar, guaranteeing both the wine's quality and its character.

What is malolactic fermentation?

For the most part, malolactic fermentation is achieved by *Oenococcus œni*, then, to a lesser degree by *Lactobacillus plantarum*, *Lactobacillus hilgardii* et *Pediococcus parvulus*. The malolactic enzyme decarboxylates the L-malic acid into L-lactic acid:



L-malic acid:1 g/L

L-lactic acid:0,75 g/L

In theory, a drop in malic acid of 1 g/L reduces total acidity (TA) by 0.4 g/L, and increases pH by 5 to 10%.

In order to control this transformation, œnological bacteria have been selected, characterized and produced on an industrial scale in line with very strict specifications in terms of purity, viability, activity and stability.

The different forms and processes for using œnological bacteria

Through the diversity of the range of bacterial preparations proposed by IOC, it is possible to fulfil both technical and economic requirements for each wine-grower.

Direct inoculation*

MBR process
direct inoculation

- EXTRAFLORE CO-IN' ™
- EXTRAFLORE COMPLEXITY ™
- EXTRAFLORE PURE FRUIT ™

Inoculation with 1 stage of acclimatisation

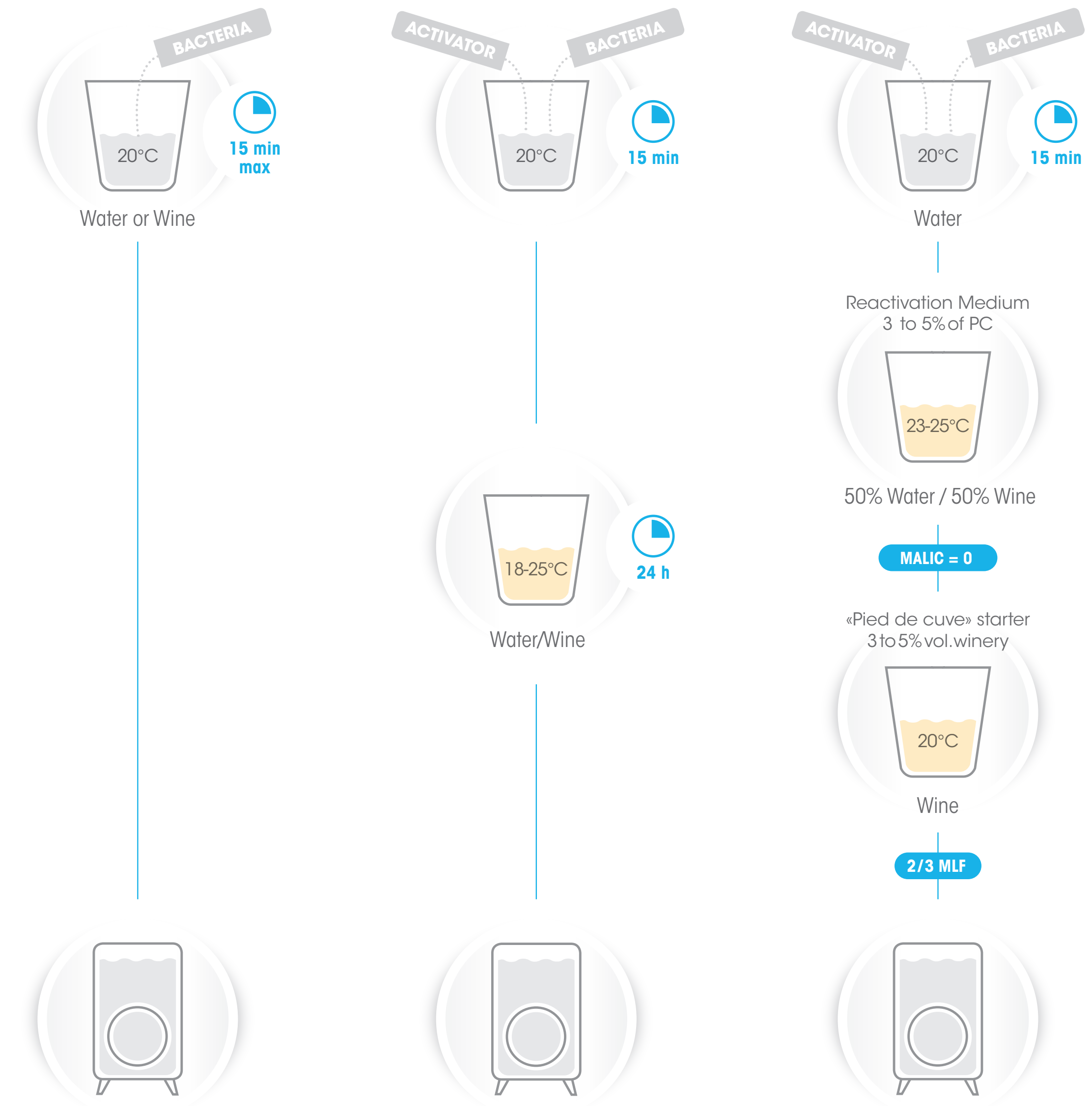
1-STEP®

- MAXIFLORE ELITE ™
- MAXIFLORE SATINE ™

Inoculation with 1 phase of reactivation and 1 starter («pied de cuve») phase

Standard

- INOBACTER™



*Placing in prior suspension is preferable in order to ensure good dispersion of the population in the wine, but direct inoculation of the tank is also possible with good homogenisation.

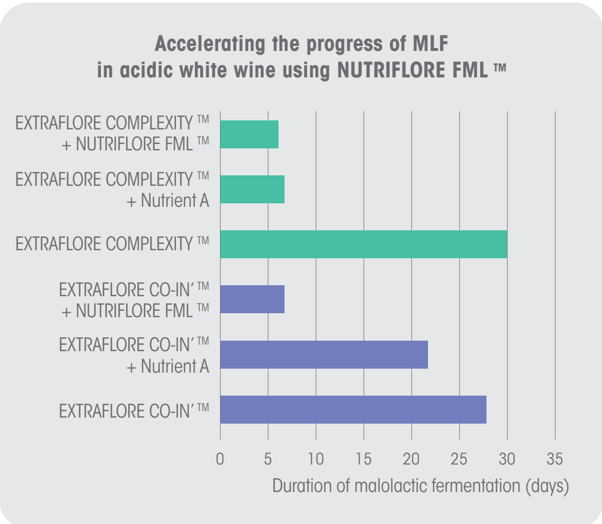
Why not launch MLF when you want?

Waiting for MLF is no longer something which is inevitable and is even not recommended, since waiting could lead to:
- costs in terms of heating and/or analytical monitoring,
- development of flora which would delay MLF,
- failure to respect the time frames for marketing or presenting wines.

Using our œnological bacteria frees you from such contingencies.

Examples of wines concerned by difficulties due to spontaneous malolactic fermentation

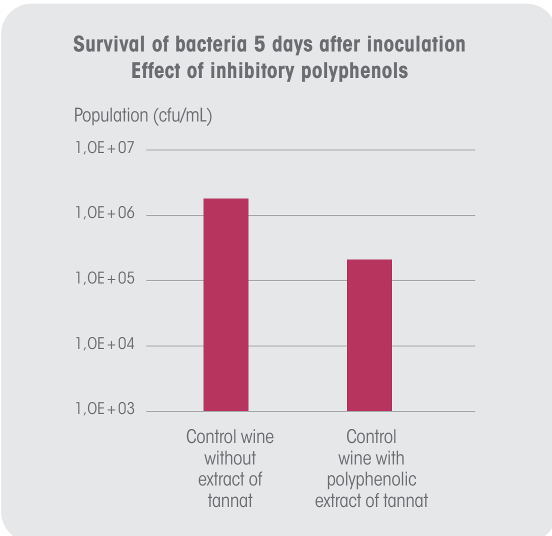
Acidic wines: excessive acidity (pH < 3.2) is more often than not the reason why it is impossible to start up MLF spontaneously. There are actions levers which can counter this:
- certain isolated œnological bacteria in white wine form a quite different genetic group that is resistant to low levels of pH,
- implementing proven and approved acclimatisation protocols,
- using nutrients which are rich in specific peptides that foster survival in acidic conditions (Bou et *al*, 2014).



Red wines from ripe grapes with high alcohol content: these wines combine a major inhibitor of bacterial activity (ethanol) at often very low contents of nutritional elements (amino acids, minerals and vitamins). In which case the following must be implemented:
- a selection of ethanol-resistant bacteria,
- use of specific nutrients.

Red wines from varieties rich in inhibitory polyphenols: recent research has shown the essential impact of certain polyphenolic fractions in blocking the activity and survival of lactic bacteria:
- existence of refractory grape varieties (merlot, tannat, etc.),
- the sometimes-negative impact of thermovinification.

Our solution :
- Raising inhibition through yeast polysaccharides (Lonvaud, 2013),
- Selecting the most efficient nutrients.



Choose the best œnological bacteria depending on the difficulty encountered

Acidic wines

NUTRIFLORE FML ™

+

pH < 3,05	3,05 < pH < 3,2	3,2 < pH < 3,3
INOBACTER™	INOBACTER™	MAXIFLORE ELITE ™
+ NUTRIFLORE PDC ™		EXTRAFLORE PURE FRUIT ™
		EXTRAFLORE COMPLEXITY ™

NUTRIFLORE FML ™ — 48hrs before adding bacteria

Nutrient improving survival in acidic conditions (rich in specific peptides)

And/Or

NUTRIFLORE PDC ™

Enhanced growth / adaptation during starter phase in acidic conditions

INOBACTER™

The safest solution for very acidic wines (pH < 3,1)

- Selected in acidic liquor (base wine)
- Preparation controlled by the microbiology laboratory of the CIVC's Direction Qualité et Développement Durable
- Standard — Acclimatization by starter phase

Concentrated wines

14,5% vol. < Alcohol < 16% vol.

co-inoculation

- EXTRAFLORE PURE FRUIT ™
- MAXIFLORE SATINE ™

sequential inoculation

- NUTRIFLORE FML ™

+

- MAXIFLORE SATINE ™
- EXTRAFLORE PURE FRUIT ™

Securing the approach of recalcitrant wines:

The predictive laboratory test

48-HOUR MALOTEST

Inoculate a sample of your wine with ten times the classic dosage of bacteria and evaluate the percentage of deterioration of malic acid after 48 hours.
If > 60% : the bacteria is suitable for your wine

EXTRAFLORE PURE FRUIT ™

The direct solution for very ripe harvests

- Triggers MLF rapidly even in the case of weak malic acid
- Very good tolerance to ethanol
- MBR process direct inoculation

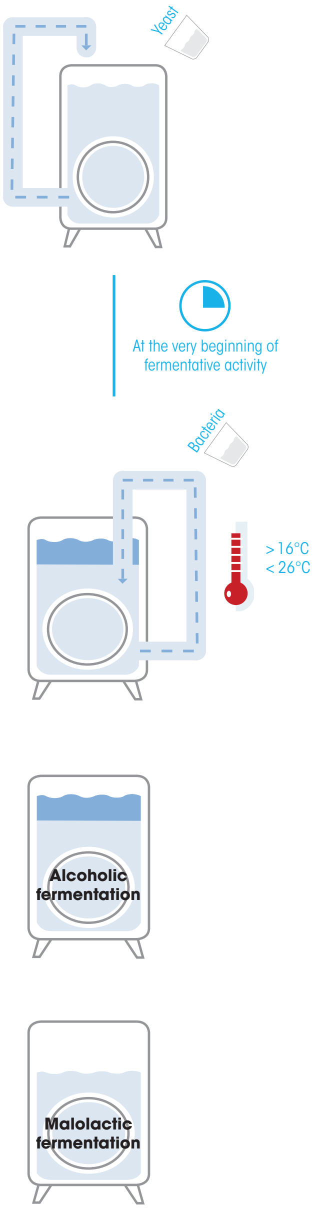
MAXIFLORE SATINE ™

The selection resistant to high contents in alcohol and inhibitory polyphenols

- Selected for its strong tolerance to ethanol
- Limited nutritional needs
- 1-STEP®

For a successful yeast/bacteria co-inoculation

Co-inoculation is today widely used by wine producers. Although the operation is easy to carry out, certain key points need to be respected. Below, we set out the protocol corresponding to a genuine co-inoculation, with close yeast and selected bacteria inoculation, as opposed to early inoculation (sometimes inaccurately referred to as «late co-inoculation»), which only introduces bacteria at the two-thirds stage of alcoholic fermentation.



1. Must/harvest: recommended **sulphiting** operation < 6 g/hL.
2. **Yeast inoculation** : choose and rehydrate a yeast that is well-adapted to the fermentability of the must and produces little SO_2 (e.g.: IOC PRIMROUGE™, IOC R 9008™, IOC BE FRESH ™, IOC BE FRUITS ™, IOC INFINI'TWICE ™…). Homogenize after inoculation.
3. At the very beginning of the fermentation activity (without necessarily waiting for a drop in density – the SO_2 simply has to combine), **inoculate the must in lactic bacteria**, under the cap of marc for red harvests. Homogenize after inoculation away from air.
4. The must **temperature** has to be between 16 and 26°C maximum, up to the end of malolactic fermentation.
5. Regular **control** of the breakdown of malic acid. Implement good yeast nutrient practices (avoid using ammoniacal nitrogen).
6. When malic acid is < 0.2 g/L: check for **volatile acidity** regularly if sugar remains.
7. **With sugar present:** if the increase in volatile acidity is around 0.1g/L per day, slightly sulphite the wine (1 to 2 g/hL) or stabilize it where lactic bacteria are concerned with suitable formulations. Early de-vatting may sometimes be recommended to facilitate homogenisation.
8. **At the end of alcoholic fermentation**: fill up the tank.
9. Generally speaking, **de-vat/rack and stabilize** wine depending on the desired itinerary.

NB – with acidic white wines (pH < 3.2): strict co-inoculation is not generally recommended because of the transient decrease in pH at the beginning of alcoholic fermentation. Wait until the two-thirds phase of fermentation has been reached before carrying out bacterial inoculation.

What if MLF were the best form of biocontrol for your wines?

For a long time, it was considered that reduction in the fruity character of a wine following malolactic fermentation was inevitable. In reality, these losses of fruitiness are the result of aromatic «masks», in particular produced by certain microorganisms. Our œnological bacteria have been selected to act preventively as biocontrol agents against such deterioration.

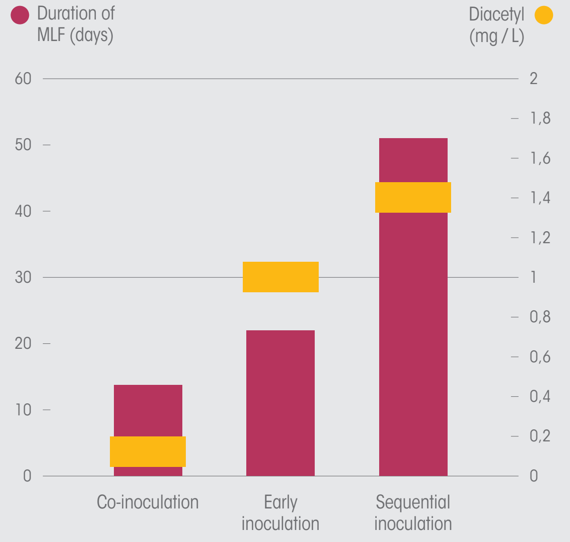
What masks and defects can œnological bacteria help prevent and why?

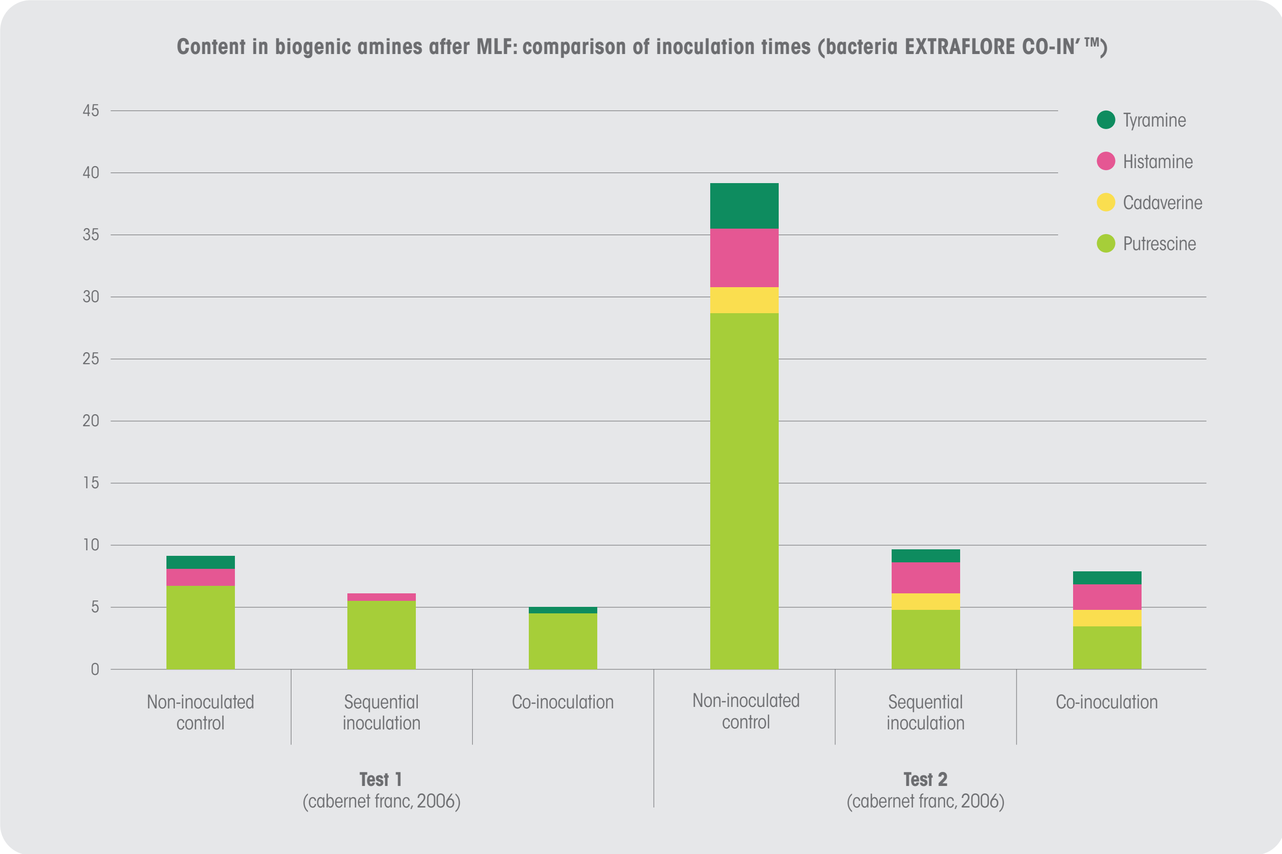
Oxidation and deviations due to too much aeration: an MLF which is late in starting after alcoholic fermentation, is a potentially unprotected wine, in particular from an oxidative point of view. Co-inoculation with œnological bacteria eliminates this risky time lapse between the end of AF and the beginning of MLF.

Excessively buttery notes: these are due to lactic bacteria (spontaneous fermentations) and may be avoided via:

- co-inoculation (fosters breakdown in diacetyl),
- certain selected bacteria (production of low amounts of diacetyl or none at all).

Duration of malolactic fermentations and production of diacetyl according to the time when bacteria are inoculated (EXTRAFLORE CO-IN' ™ - chardonnay 2010)

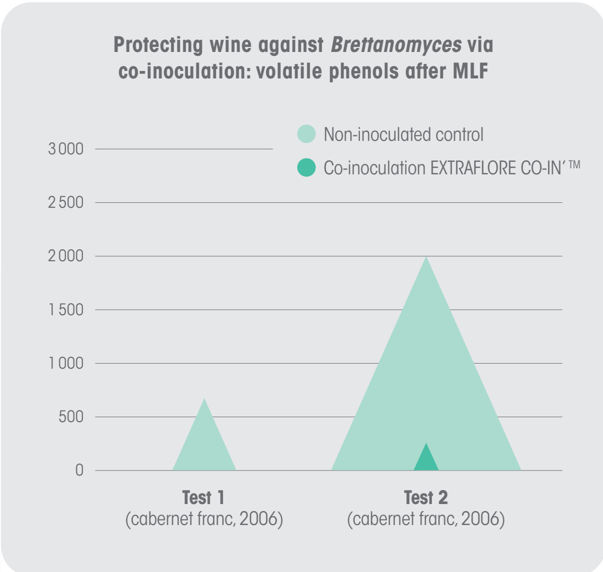




The «biogenic amines» mask: often produced by indigenous bacteria, volatile biogenic amines (putrescine, cadaverine) can mask fruity aromas (Palacios et *al*, 2005). Our œnological bacteria are incapable of releasing such. The earliest modes of inoculation are ideal for reducing risks (Pillet et *al*, 2007).

Phenolic tastes: *O. oeni* bacteria have proven biocontrol power with regard to *Brettanomyces* including after MLF. Our œnological bacteria are also unable to produce precursors of volatile phenols.

Bacterial deviations: lactic spoilage and acetification, ropiness, mousiness..., there are a host of potential defects caused by uncontrolled bacterial activities. Controlling malolactic fermentation through selected microorganisms is undisputedly the best way to eliminate these alterations.



Choosing the best œnological bacteria for biocontrol according to the risk of deterioration

Pot^{al} alcohol < 13,5% vol.	Pot^{al} alcohol > 13,5% vol.
co-inoculation **EXTRAFLORE CO-IN' ™**	co-inoculation preferably otherwise 2/3 AF **EXTRAFLORE PURE FRUIT ™** **MAXIFLORE SATINE ™**

Later bioprotection (post-AF) remains possible with **EXTRAFLORE PURE FRUIT ™** or **MAXIFLORE SATINE ™**

EXTRAFLORE CO-IN' ™

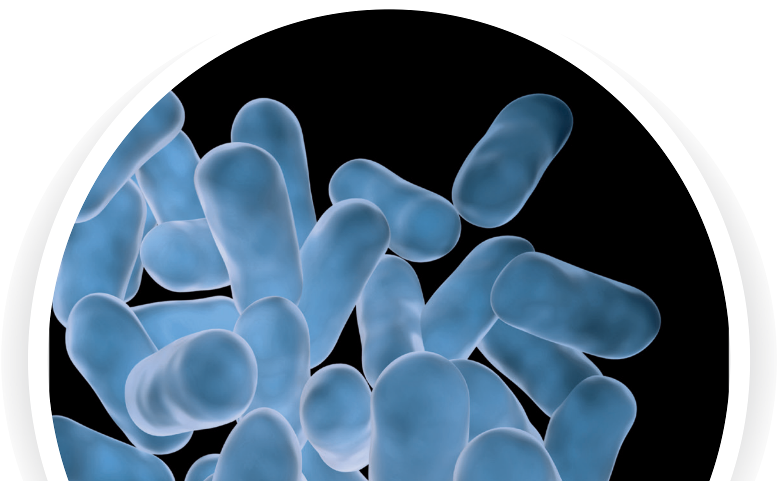
Prevents microbial development in co-inoculation

- Specifically for co-inoculation at the beginning of AF
- POMACE / MUST – Suitable for winemaking with medium- to long maceration
- *MBR process* *direct inoculation*

MAXIFLORE SATINE ™

Co-inoculation and early inoculation in more restrictive conditions

- Great robustness (alcohol, polyphenols, nutrients...)
- May also be used in early inoculation (2/3 AF)
- POMACE / MUST – Suitable for winemaking with medium- to long maceration

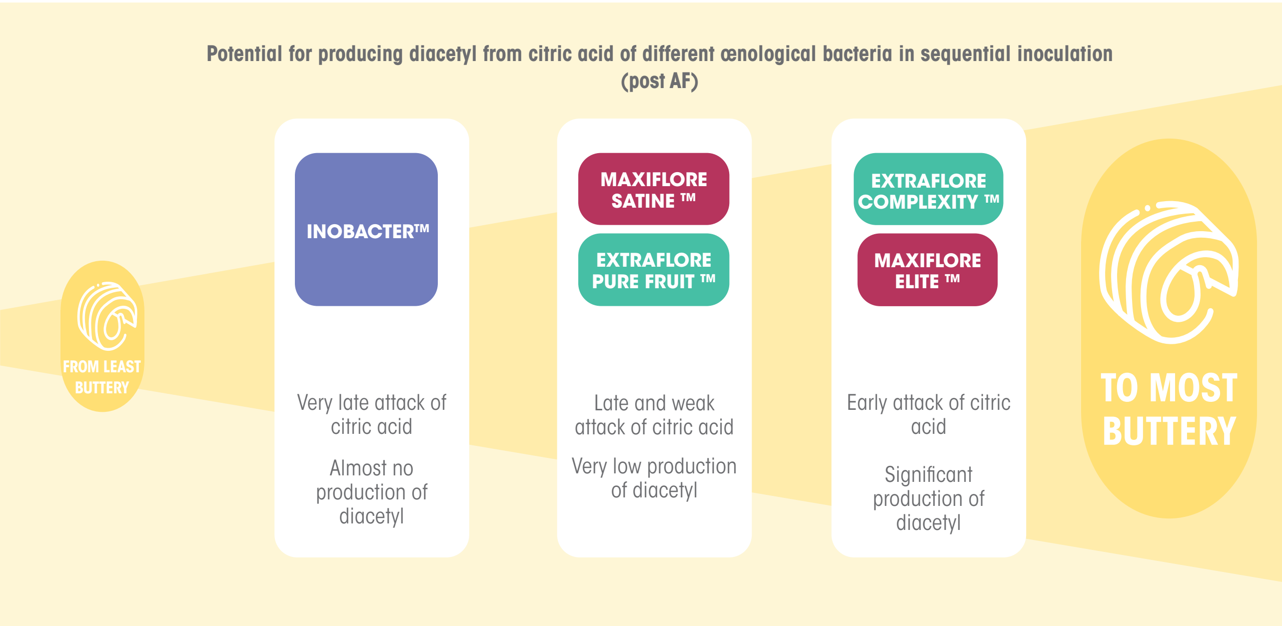


Why not use MLF for sensory enhancement?

For a long time denied in œnology, the sensory impact specific to each lactic bacteria is today an unchallengeable reality. There is increasing scientific and technical proof provided by the work carried out in numerous research institutes.

How can an œnological bacteria influence the style of a wine?

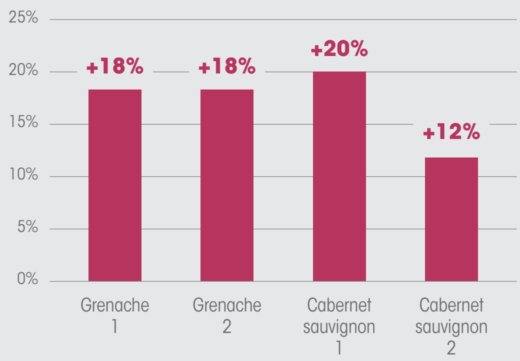
Buttery notes: impact of the choice of bacteria and/or the time of inoculation (co-inoculation fosters a reduction in buttery notes).



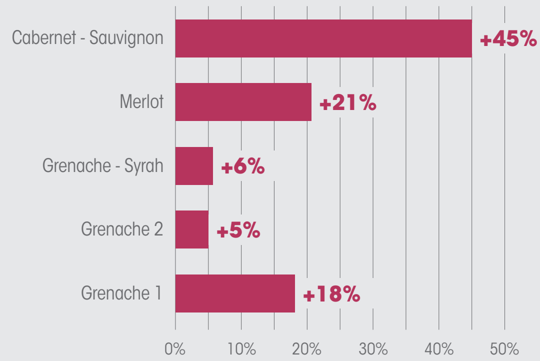
Notes of red and black fruits: depending on the activities inherent in each strain, lactic bacteria may produce but also deteriorate fruity acetate esters and fatty acids (Bartowski et *al*, 2009; Knoll et *al*, 2011).

Terpene aromas of spices and flowers: depending on the bacteria, the glycosidasic activity contributes more or less to releasing terpene aromas which give red wines spicy, resinous or even flowery aromas.

Phenylethyl acetate: benefit (%) obtained with MAXIFLORE SATINE ™ bacteria against MAXIFLORE ELITE ™ bacteria



Linalol : benefit (%) obtained with MAXIFLORE ELITE ™ bacteria against MAXIFLORE SATINE ™ bacteria

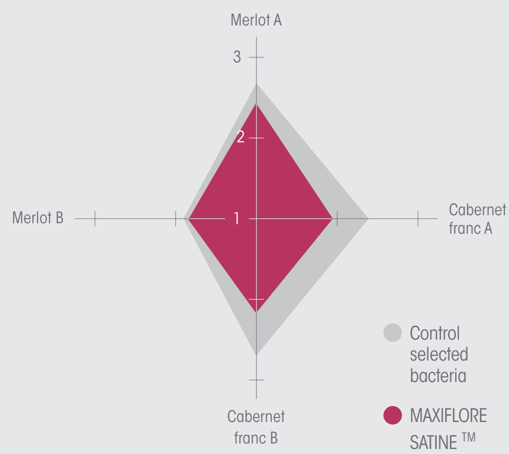


The herbaceous character: certain bacteria may deteriorate hexanal and hexanol (herbaceous aromas) and are capable of transforming fatty acids (herbaceous odours) into fruity esters.

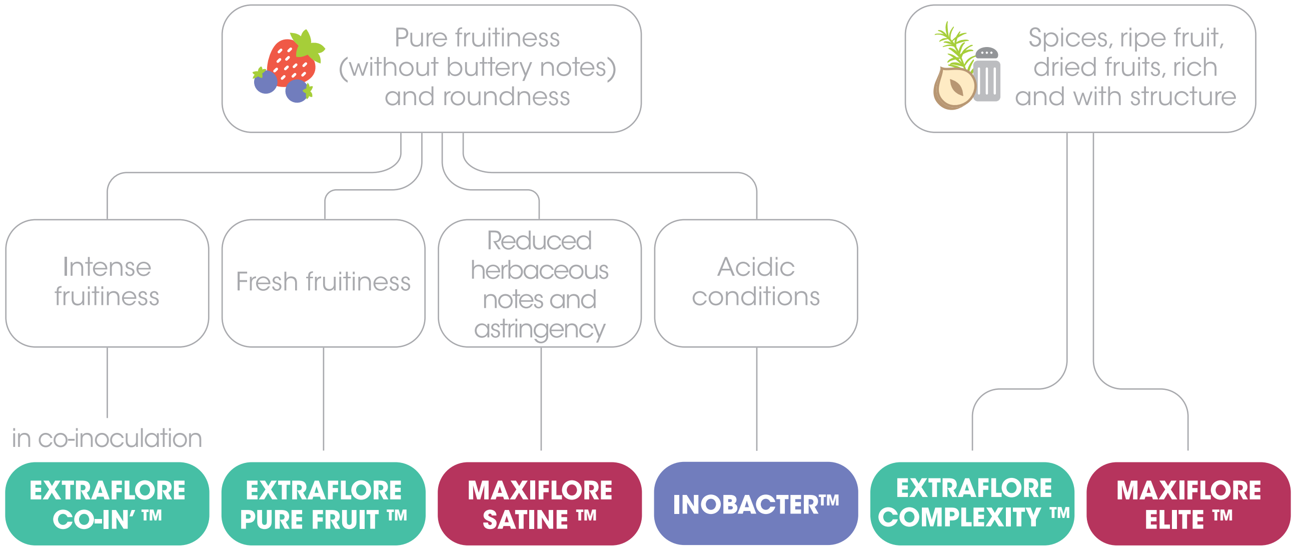
Woody notes: the enzymatic activities of certain bacteria release aromatic compounds from barrels or alternatives (Bartowsky et Hayasaka, 2009).

Astringency and full-bodiedness: our works show that some of our œnological bacteria contribute to roundness and reduction of astringency in wines.

Intensity of astringency: sensory analysis on test wines



Choosing your œnological bacteria to differentiate your styles of wine



EXTRAFLORE CO-IN' ™

Brings out and preserves fruity esters

- Dedicated to co-inoculation at the beginning of AF
- Contributes strongly to fruity esters
- Very low production of diacetyl (in co-inoculation)
- MBR process direct inoculation

EXTRAFLORE PURE FRUIT ™

Fruity freshness and balanced on the palate

- Very low and late production of diacetyl: no masking of fruitiness by butter/milk
- Contributes to fruity freshness (esters)
- Roundness and Suppleness
- MBR process direct inoculation

MAXIFLORE SATINE ™

Purity of fruit and reduced greenness

- Very low and late production of diacetyl: no masking of fruitiness by butter/milk
- Aromas of flowers and red and black fruits
- Consumption of hexanol and limitation of herbaceous notes
- Roundness and reduction of astringency
- 1-STEP®

EXTRAFLORE COMPLEXITY ™ / MAXIFLORE ELITE ™

Aromatic complexity and structure

- Production of 2-phenethyl and terpenols (floral, spicy and balsamic aromas)
- Production of diacetyl which could overshadow certain herbaceous notes and contribute to notes of toast and dried fruit
- Highlights structure and full-bodiedness of red wines
- MBR process direct inoculation
- 1-STEP®

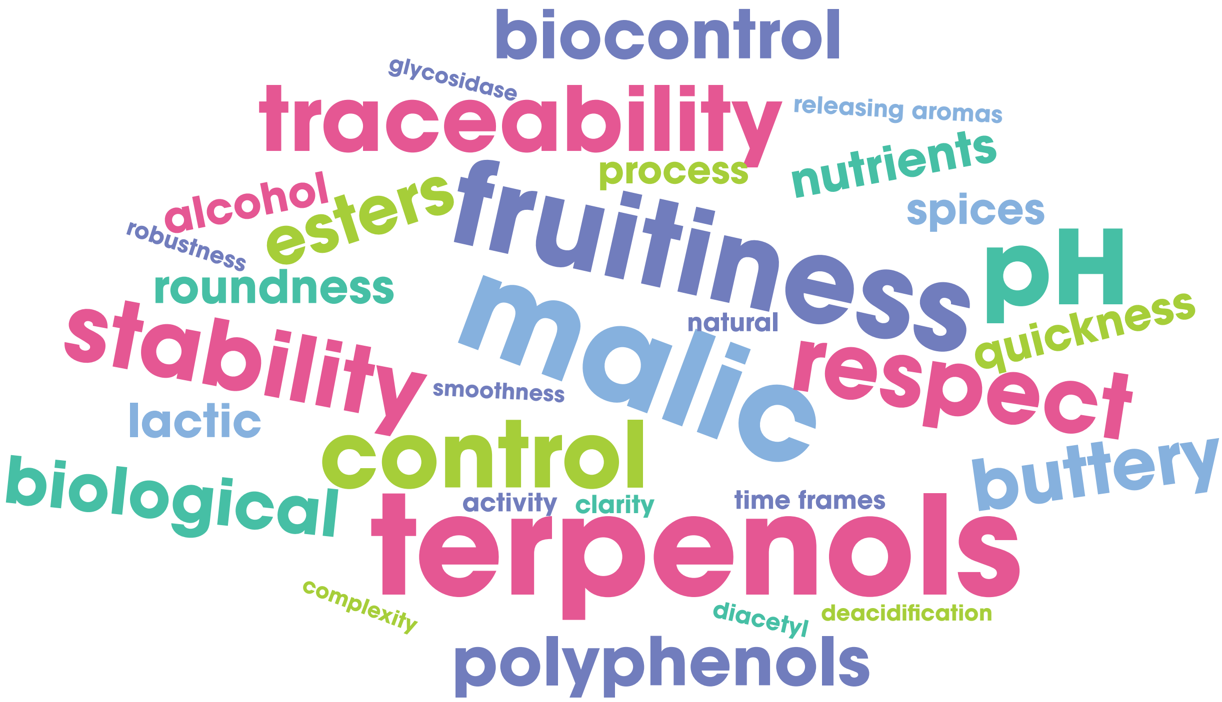
Technological properties and fields of application of our œnological bacteria

		EXTRAFLORE CO-IN' ™	EXTRAFLORE COMPLEXITY ™	EXTRAFLORE PURE FRUIT ™
Range of use	Type of product	MBR process direct inoculation (direct inoculation)	MBR process direct inoculation (direct inoculation)	MBR process direct inoculation (direct inoculation)
	Ease-of-use	★★★★	★★★★	★★★★
	Co-inoculation	★★★★	★	★★★
	Sequential inoculation	★	★★★★	★★★★
	Maximum alcohol	< 13,5% vol.	< 14% vol.	< 16,5% vol.
	Minimal pH	> 3,25	> 3,2	> 3,2
	SO_2 total max.	< 60 mg/L	< 40 mg/L	< 50 mg/L
	Temperature	18-26°C	18-26°C	15-26°C
	Polyphenol resistance	★★	★★	★★★
Sensorial profile	Aromatic complexity	★★★★	★★★★	★★
	Diacetyl (buttery)	Null in co-inoculation	Medium	Very low
	Spices	★	★★★★	★
	Fruitiness	★★★★	★★	★★★★
	Roundness	★★	★★	★★★
	Structure	★	★★★★	★★
Œnological application	White wine	★★	★★	★★
	Red wine	★★★★	★★★★	★★★★
	Rosé wine	★★	★	★★★
	Base wine	★	★	★
	Early «primeur» wine	★★★★	★	★★★

MAXIFLORE SATINE ™	MAXIFLORE ELITE ™	INOBACTER™
1-STEP® (fast acclimatization)	1-STEP® (fast acclimatization)	Standard («pied de cuve» starter)
★★★	★★★	★
★★★★	★★	★★
★★★★	★★★★	★★★★
< 16% vol.	< 15,5% vol.	< 13,5% vol.
> 3,25	> 3,2	> 2,9
< 60 mg/L	< 60 mg/L	< 60 mg/L
18-26°C	18-26°C	16-20°C
★★★★	★★	

★★	★★★★	★★★
Very low	Important	Very low
★★	★★★★	
★★★	★★	★★★
★★★★	★★	★★★
★★★	★★★★	

★★	★★	★★★★
★★★★	★★★★	★
★★	★	★★
★	★	★★★★
★★	★	★



Find the decision support tool on our website to help you choose the oenological bacteria and protocol best suited to your situation and your management of malolactic fermentation.

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